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Bridging Pathology and Toxicology: The Expanding Role of Veterinary Toxicopathologists in Regulatory Science

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Introduction

Regulatory toxicology is a rapidly growing field in toxicology and it is highly influenced by advancement in science and technologies. Events such as death caused by cough syrup containing sulfanilamide in ethylene glycol (1938) and thalidomide tragedy (1960) result in the formation of modern regulators in Toxicology. The regional, national and global safety regulations follow the good laboratory practices (GLPs), good manufacturing practices (GMPs) and good clinical practices (GCPs). Since beginning, pathology has been a main stream in all regulatory studies using experimental animals and later emerged as a separate entity "Toxicologic Pathology". It interlinks the disciplines of toxicology and pathology. Toxicologic pathologist mainly works in identification and interpretation of substance induced health effects on humans and animals. They have been extremely helpful in underpinning of decisions like NOAEL (No Observed Adverse Effect Level) of agents in toxicology and differentiation of various pathological changes like alteration and adaptive changes due to toxins, spontaneous and degenerative lesions[1]. Majority of toxicologic pathologist are working in industrial settings for pre-clinical toxicity or non clinical safety assessment of pharmaceutical agents, pesticides, food additives, or contaminants. Being a member in the multidisciplinary team for safety testing of drugs and chemicals, a toxicologic pathologist interacts with the study sponsors, toxicologists, animal resources, clinical pathology, peer reviewing pathologist, management and regulatory agencies.

Regulatory Toxicologic Pathology (RTP) is a vitally important professional practice of pathology as applied to Regulatory toxicology. It involves morphological and functional evaluation of organs/tissues collected during safety studies. In industry, comparative studies of chemical exposures between model organisms and humans are important for the risk assessment of chemicals and human health. Regulatory toxicologic pathologists play a key role to determine the nature and characterization of hazards and participate in all aspects of the toxicity studies including study design and data generation. Pathologists contribute in defining NOAEL, based on the extent of severity of lesions to the level of exposure, collecting historical control findings, development of alternative models as well as alternative markers in the field of regulatory toxicology. All these become a valuable input in formulating regulatory guidelines for efficient development of a product. In a GLP (Good Laboratory Practice) accredited laboratory, a pathologist has certain standard role as a study pathologist, peer review pathologist/ pathology working group where they evaluate the gross and microscopic changes induced by a chemical/compound and submit the pathological

report to study director after discussion and evaluation of the data. A study pathologist, review the rata data (haematology, biochemical analysis, organ weights, feed consumption, clinical signs and body weight) of study under evaluation. Histopathological data of control and high dose groups were evaluated, if any lesions noticed in high dose, then low and mid dose groups were also observed. The pathology report is ultimately reviewed by a peer review pathologist and 10% of the slides screened randomly. All the study data and interpretations in agreement with both the pathologist were duly signed by a study pathologist and submit it to the study director. If the observations of peer pathologist not matching with the study pathologist observations, then the slides were evaluated by pathology working group.

Toxicologic pathology is practiced as a subspecialty, mainly by veterinary and medically qualified pathologists across the globe. In India, large proportion of the pathologists working in the field of toxicology is Veterinary toxicologic pathologist. Most of them are employed by industry, including bio/pharmaceutical and chemical companies, as well as contract research organizations (CROs). The concern of U.S FDA regarding the neurotoxicity risk assessment was rectified by pathologists by improving detection techniques like immunohistochemistry, fluorescent microscopy rather than increasing animal usage. U.S FDA has drafted the use of histology as a guidance document for industry. Society of toxicologic pathologist (STP) committee provided feedback to U.S FDA that the biomarker qualification not recommended for GLP studies unlike non GLP studies. Another example is veterinary toxicologic pathology community intended the use of recovery group animals in cancer therapeutic studies. The European Union (EU) regulations on the safety of cosmetic ingredients/cosmetics forbid animal testing in support of 3Rs. Hence, we can conclude that the toxicologic pathologist have a significant role in regulatory policy. These approaches shifted the toxicologic pathology field into the direction of adapting to new science by following the principles of 3Rs (Replacement, Reduction and Refinement). Industries and regulatory bodies were interested in conducting toxicity studies based on 3Rs principles and need pathologists to practice the discipline on internationally accredited quality platforms; enhanced by applying molecular pathology with advanced observation techniques.

In pre-genomic era, the animal experiments in toxicologic pathology were focused on studies of cancer induced by the toxicants and it led to the development of the two-year rodent cancer bioassay by toxicologic pathologists in the United States. Invariably in the current genomic era, with the development of omics revolution and high throughput screening, new specialized animal models such as outbred mice, knockout mice etc were developed by genetics community. Only a skilled toxicologic pathologist can conduct the morphological and functional phenotyping of the newly developed models and evaluate their performance in toxicity and safety evaluation studies. Predictive toxicology based on database in post genomic era, the computerized algorithms predict the adverse effects of chemicals/drugs and it needs confirmation using *in-vivo* models. Toxicologic pathologists will work without much changes in the post genomic era where they have to see actual tissue morphological features in test species to identify the actual test item and interlink it with the *in silico* data mining and its probability predictions. Since pre-genomic era, most of toxicologic pathology training has occurred “on the job”. Specific training on toxicologic pathology is lacking in both veterinary and medical educational programs. In reality, no university in the country has any formal higher education or training program in the subject, so as so the toxicologic pathology is a self-trained or on-job-acquired specialty skill rather than earned-skill at academic settings. After MAD (Mutual Acceptance of Data) agreement with OECD (Organization for Economic Co operation and Development), India become a rapidly expanding platform for pre-clinical research which needs lot of manpower to perform toxicologic pathology studies. This emphasizes the need of training centers to create awareness and train the toxicologic pathologists about the new and emerging field, so that the future toxicological pathologist will be able to prepare for

future challenges and stay relevant in the field of toxicologic pathology.

The future of toxicologic pathology is digital pathology and Artificial Intelligence (AI). Digital pathology has expanded in all aspects of pathology including regulatory toxicologic pathology in primary histopathology reads and peer review. Pathologists play a key role in the process of converting histopathology glass slides into digitization and data generation using suitable algorithms. Digitization of pathological slides using Whole slide Imaging (WSI) technology has become a standard procedure in telepathology/telemedicine. At present we are in the ground zero level. WSI convert glass slide pathology data into high resolution virtual image and stored as digital files. Instead of photographing of specific region, the entire slides are digitized using WSI scanner. Continuous technology advances made microscopes of high resolution and high through put scanners. WSI facility in pathology laboratories has innumerable advantages in diagnosis, education and research. Telepathology using WSI is validated in centralized pathology laboratories for seeking an expert opinion in surgical pathology, cytopathology, immunohistochemistry and electron microscope. Online WSI is used for learning and training purposes. For researchers along with tissue microarray, WSI helps to scan a number of biomarkers at a time. Although there are advantages, the cost of machine and validation of the techniques and lack of expertise are limiting its application in laboratories.

In regulatory studies, WSI can be used in Quality assurance review and archiving facility. Use of this technique in Quality Assurance (QA) program will help in reducing the peer review bias. Physical storage space for the H&E-stained glass slides and loss of stored slides quality, difficulty in searching is some of the issues in archival facility. By using this digital technique, the pathology data are stored in high resolution digital files, anyone can access the data at any time, no loss in sample clarity, it become easy to archive, easy to search and the physical storage space also reduced. The U.S. FDA recommends the whole slide image files should be retained along with the glass slides as study records and archived after study finalization. Using WSI in GLP compliance regulatory laboratories require adequate documentations, SOPs for whole slide imaging processes like slide scanning, validation, training, maintenance, software version control, backup recovery, virus protection, archival, secure access controls and data transmission are required. Signed pathology report should state that the whole slide images were evaluated instead of glass slides. The histopathology data can be shared among the study pathologists, study director, peer review pathologists. After discussion of the pathological data, a formal peer review report signed by the study pathologists and peer review pathologists in agreement will be submitted to any regulatory agencies. Digital images, if linked with the final study report can be sent to sponsors and it will increase the clarity that the toxicology studies are typically read in an open fashion, and similarly peer reviewed to avoid the false positive signals. Online viewing, annotation, and dialogue between pathologists, study director, sponsors and regulators facilitates good science and economic benefits by enabling more timely and informed clinical decisions. Practicing of this facility in GLP compliance esp. in government regulations may require validation of digital pathology systems. Although the technique is faster, initial implantation cost is high. Archiving, data retrieval and access right for sponsors and regulators are some other regulatory issues.

WSI tool in digital pathology is the pioneer for telepathology and artificial intelligence (AI) in pathology. Application of telepathology in regulatory studies is to mainly co-ordinate the interdisciplinary team members in a preclinical toxicity study by sharing the pathology data that can access at anytime and can involve in discussion from anywhere. Toxicopathology labs of different CROs in a country can consult each other for peer review and can collect data using the modern telepathology system. Moreover, such control data will be used to validate AI models in pathology. This will also help to eliminate expensive repetitions of testing compounds and expedites the regulatory decisions. Modern telepathology can also make use for conducting webinars. Instead of photographing a

portion/section, the whole slide is digitalized in WSI and it becomes the basis for data archive. Ability and robustness of AI depends on the amount of underlying data. The data buildup is possible only by storing historical control animal data used in toxicity studies and by online collection of histopathological slide data anywhere anytime. The potential of AI in pathology is mainly to create image analysis tools such as tumor grading, immunohistochemistry scoring and quantitative histomorphometry. Image analysis tools are widely used in preclinical studies to quantify cells and nuclei using traditional image analysis methods and organ quantification using machine learning. Not only H&E slides, the protein expression in immunohistochemistry slides were also quantified using image analysis tool. The AI models were generated for image analysis and used successfully in screening bone marrow cells and neuronal cells of laboratory animals used in toxicity studies. Continuous generation of new AI models with different training data is extremely needed in this century for the easiness of pathologists. The quality of data generated using digital image analysis in toxicologic pathology should be verified by toxicologic pathologists for implementation of the quality assurance. Hence it is evident that the pathologist plays a crucial role in data generation in the process of algorithm development and review of generated data and its interpretation. AI in pathology is mere a supportive tool for pathologist and not an alternative for toxicologic pathologist, because certain activities like necropsy, macroscopic examination of organs, examining and interpreting complex pathological data and for peer review and quality assurance of data, a toxicologic pathologist is needed.

New technologies, new products and new scientific ideas creates uncertainties and we need to have training and retraining so that the toxicologic pathologist can be geared towards the rapidly developing science. How well and how rapid we toxicologic pathologists adapt in this "Omic" era is important to determine the role of toxicologic pathologists in product development and its approval processes in the field of regulatory toxicology. The ability to evaluate both anatomic and clinical pathology data electronically will facilitate a global collaboration. It is important to train toxicologic pathologists about basic understanding of digital pathology, image analysis tools and machine learning. Designing a digital toxicopathology laboratory with all these facilities will helps in training the next generation of virtual pathologists. Digitalization can't replace pathologist's knowledge, but it can support and accelerate pathologist work for making better regulatory guidelines to safeguard the health of public, consumers, workers and environment.